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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant : Charles E. Clum et al.
Serial No.: 700,165 Group No.: 125
Filed : February 11, 1985 Examiner : J. Lipovsky
For : SKIN CARE COMPOSITIONS

DECLARATION UNDER RULE 132

Honorable Commissioner of Patents and Trademarks
Washington, D. C. 20231

Dear Sir:

I, DAVID M. ISAACSON, declare and say:

THAT I received a B.S. degree in Psychology from the University of Pittsburgh in 1948, a M.S. degree in Microbiology from the University of Pittsburgh in 1952, and a Ph.D. degree in Microbiology from the University of Pittsburgh in 1964;

THAT I have authored various papers and delivered various lectures in areas of microbiology and further that I have authored various articles and chapters in books on analytical microbiology;

THAT I have been employed as a microbiologist since 1952;

THAT I was employed from 1952 to 1958 as a microbiologist, chief analyst and laboratory director for the Department of Health, City of Pittsburgh;

I was employed from 1958 to 1961 as a microbiologist for Nuclear Science & Engineering Corp., Pittsburgh;

THAT from 1961 to 1964 I was a teaching assistant at the University of Pittsburgh while completing my doctoral studies;

THAT from 1964 to 1975 I was employed as a microbiologist for E. R. Squibb & Sons, New Brunswick, NJ;

THAT from 1975 to the present I have been employed by Johnson & Johnson and presently am a Principal Scientist in microbiology for the Ortho Pharmaceutical Corp. subsidiary of Johnson & Johnson;

THAT I am an inventor of and familiar with the invention disclosed and claimed in United States Patent Application Serial No. 700,165 and further that I am familiar with the Office Actions and prior art relating to said application and its parent application;

THAT I performed and supervised experiments in accordance with the procedure set forth on pages 10-12 of the specification of United States Patent Application Serial No. 700,165. Generally, this consisted of preparing weighed suspensions of zinc oxide and/or miconazole nitrate in measured volumes of melted microbial growth supporting agars. Aliquots of these agar suspensions were transferred to petri plates and allowed to solidify. During the solidifying process, the zinc oxide and/or miconazole nitrate particles were maintained in suspension in the agar in the petri plates by imparting constant motion to the plates positioned on a reciprocating or rotary platform shaker. To the surface of the solidified agar suspension was added 0.02 ml of inoculum of S. aureus containing approximately 300 colony forming units (CFU) of microbial suspension (1.5×10^4 CFU/ml). The organisms were then distributed evenly over the agar surface with a sterile

glass spreader. The plates were then incubated 2 to 4 days at 35°C. After the incubation period, the plates were examined for evidence of growth inhibition by determining the presence or absence of surface colonies. The number of colonies on each plate at each concentration of miconazole nitrate alone was noted. Similarly the number of colonies was noted on each plate at each concentration of zinc oxide alone, and on plates containing combinations of different concentrations of both miconazole nitrate and zinc oxide. The "percent inhibition" of the bacteria produced at a particular concentration of miconazole nitrate alone was calculated by comparing the number of colonies obtained at that concentration with the number of colonies of the negative control plate containing no zinc oxide or miconazole nitrate. Similarly, the "percent inhibition" produced at one concentration of zinc oxide alone was calculated by comparing the number of colonies obtained at that concentration of zinc oxide with the number of colonies produced on the negative control plates containing no miconazole nitrate or zinc oxide. To determine the effect of zinc oxide on miconazole nitrate activity, the "percent inhibition" of the organisms in contact with any of the combinations of zinc oxide and miconazole nitrate concentrations were calculated by relating the number of surviving colonies on the agar surface of those plates with the number of surviving colonies on the base line control plates containing the same concentration of zinc oxide alone. These results are expressed as percent inhibition of the organism at a specific concentration of miconazole nitrate alone (Inhibition for Miconazole); as percent inhibition of the organism at a specific concentration of zinc oxide alone (Inhibition for Zinc Oxide); as the percent inhibition expected

if the inhibition obtained for miconazole nitrate and for zinc oxide were additive (Sum of Components), and as the observed inhibition at specific concentrations of zinc oxide and miconazole nitrate (Inhibition for Combination):

THAT when the above test procedure was utilized for a miconazole nitrate concentration of 0.60×10^{-4} W/V and a miconazole nitrate to zinc oxide ratio of 1:60 the following results were obtained:

Synergistic Activity of Zinc Oxide on Miconazole Nitrate

Inhibition of the Growth of Staphylococcus aureus at

Miconazole Nitrate Concentration of 0.60×10^{-4} % W/V

Zinc Oxide Concen- tration $\times 10^{-2}$ % W/V	Inhibition for Miconazole Nitrate	Inhibition for Zinc Oxide	Sum of Compo- nents	Inhibition for Combination
0.00	11.6	0.0	11.6	11.6
0.36	11.6	8.2	19.8	30.1

THAT when the above test procedure was utilized for a miconazole nitrate concentration of 0.80×10^{-4} W/V and a miconazole nitrate to zinc oxide ratio of 1:60 the following results were obtained:

Synergistic Activity of Zinc Oxide on Miconazole Nitrate

Inhibition of the Growth of Staphylococcus aureus at

Miconazole Nitrate Concentration of 0.80×10^{-4} % W/V

Zinc Oxide Concen- tration $\times 10^{-2}$ % W/V	Inhibition for Miconazole Nitrate	Inhibition for Zinc Oxide	Sum of Compo- nents	Inhibition for Combination
0.00	0.00	0.00	0.00	0.00
0.48	0.00	15.3	15.3	51.9

THAT the results in the above tables clearly demonstrate the synergistic activity of combinations of miconazole nitrate and zinc oxide in a 1:60 ratio against Staphylococcus aureus:


THAT in my opinion, based on the above data and data in the specification, combinations of miconazole nitrate and zinc oxide exhibit synergistic activity against Staphylococcus aureus in ratios of from about 1:60 to 1:333;

THAT in my opinion, based on the data in the specification and the additional data in the Declarations Under Rule 132 of Dr. Bruce Semple and Dr. James Leyden submitted herewith, combinations of miconazole nitrate and zinc oxide exhibit synergistic activity against Candida albicans in ratios of from about 1:60 to 1:333;

THAT in my opinion the compositions of the present invention would not be obvious to one skilled in the art.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Subscribed this 10th day of March, 1986.


David M. Isaacson

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